

GAS CHROMATOGRAPHY

by ROY A. KELLER

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GAS CHROMATOGRAPHY

A simple analytical method sharply separates components of complex mixtures. When used to characterize perfumes and flavors, its sensitivity rivals that of the human nose

by Roy A. Keller

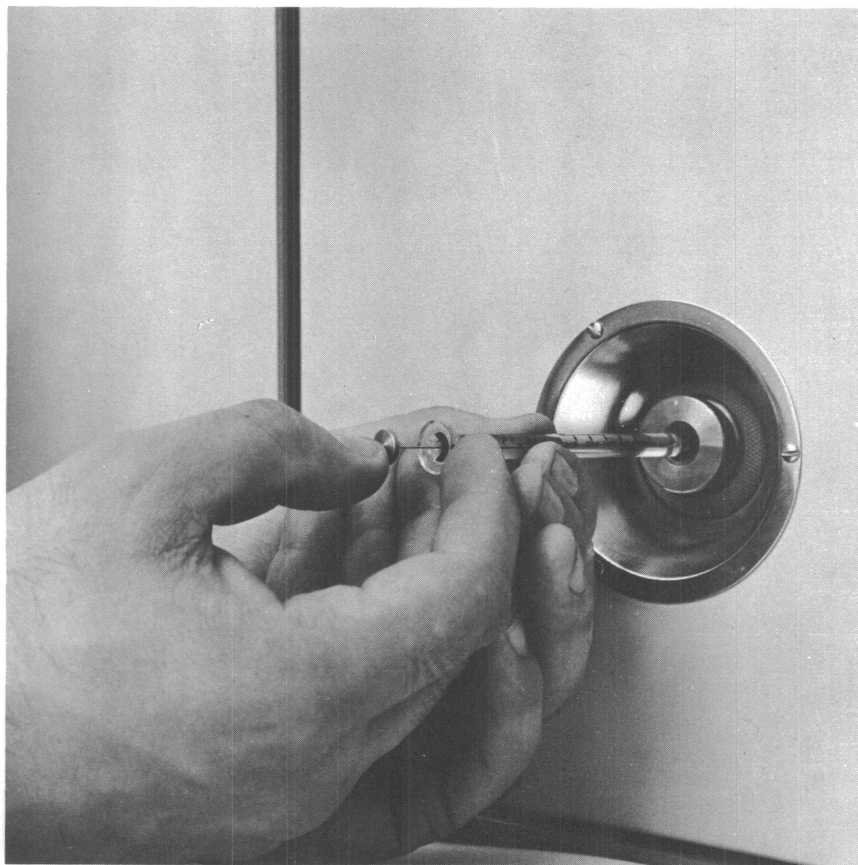
The most pervasive problem in chemistry and biochemistry is that of determining the composition of complex mixtures of matter. The research chemist would like to know at all times exactly what his test tubes and flasks contain; the industrial chemist has an equally urgent need to know the composition of materials flowing through reaction vessels and distillation columns

and into product tanks. Within the past 20 to 30 years, in response to this need for fast and accurate analyses, a number of powerful analytical tools have been developed. They include instruments that measure how compounds absorb ultraviolet and infrared radiation, instruments that determine atomic or molecular mass and instruments that determine how the magnetic properties of

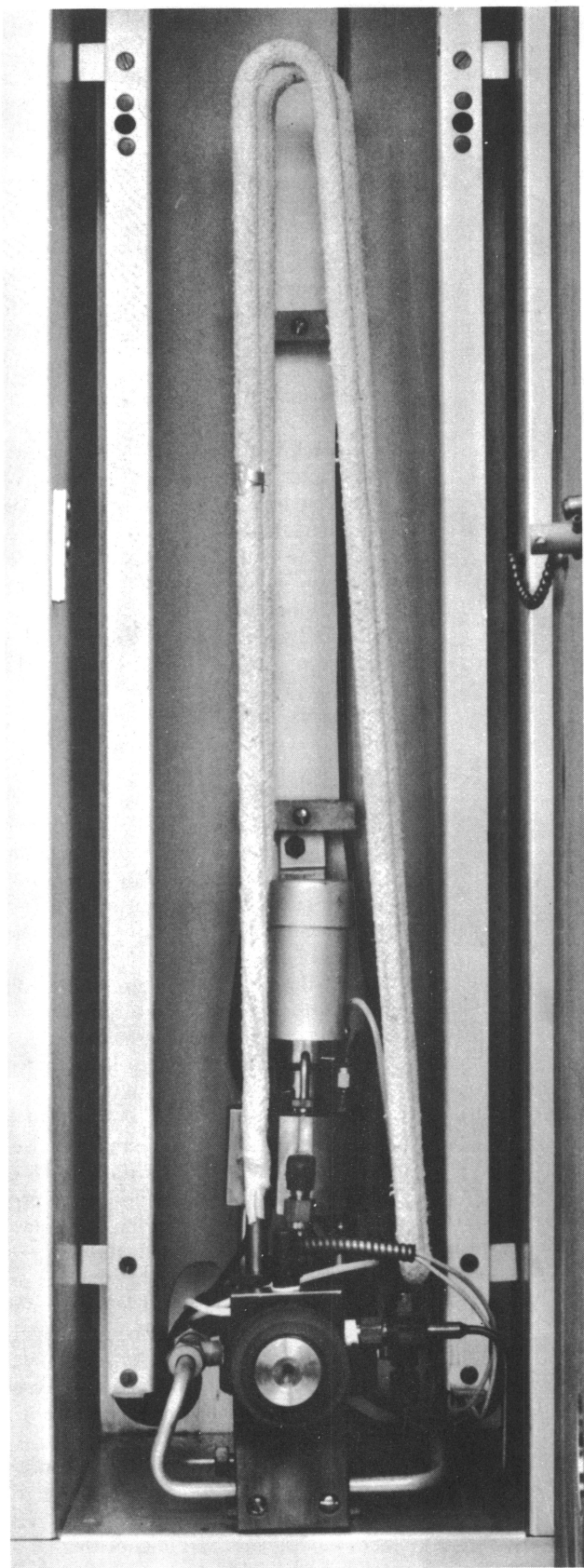
atomic nuclei are influenced by different molecular configurations.

One of the newest and most versatile analytical techniques is gas-liquid chromatography, usually referred to simply as gas chromatography. The name is somewhat misleading; there is nothing chromatic about the method or its results. The name comes from the original method of liquid-solid chromatography described in 1906 by Michael Tswett, a Russian botanist. Tswett found that when a solution of chlorophyll was allowed to filter through a column firmly packed with pulverized calcium carbonate, the various fractions of the chlorophyll mixture separated into distinctively colored bands. He called the result a chromatogram [see "Chromatography," by William H. Stein and Stanford Moore; *SCIENTIFIC AMERICAN*, March, 1951]. A useful variation of Tswett's original concept is paper chromatography, in which compounds in solution migrate at different speeds across a sheet of porous paper.

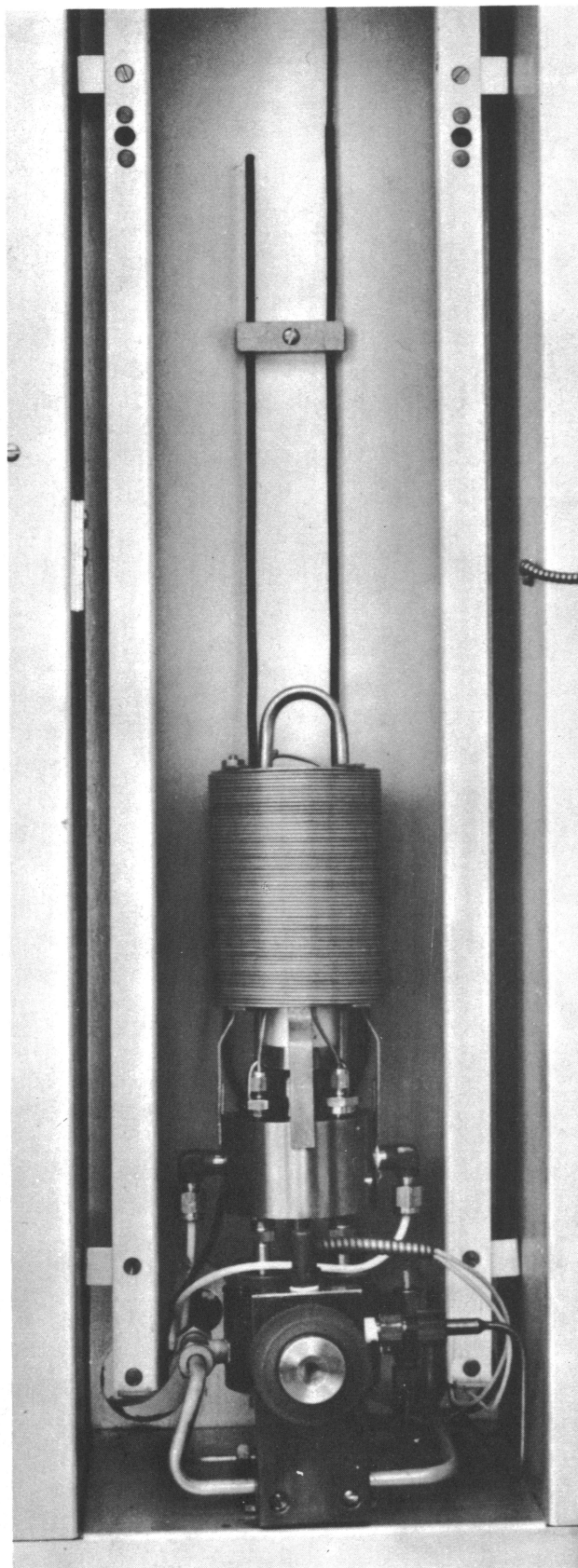
In gas chromatography the compounds in a mixture migrate at differing speeds when carried along by an inert gas through a tube that has been packed or treated in a special way. The method was first suggested in 1941 by the British chemists A. T. James and A. J. P. Martin. The method underwent development in many laboratories beginning around 1950, and the first commercial instrument came on the market in 1955. Today some 40 models of gas chromatographs are being built by 20 U.S. and a dozen European manufacturers. The instruments are widely used for analyzing complex organic mixtures such as those commonly found in petroleum products, essential oils, perfumes, flavors and other substances of biological origin. Samples containing as many as 76 dif-



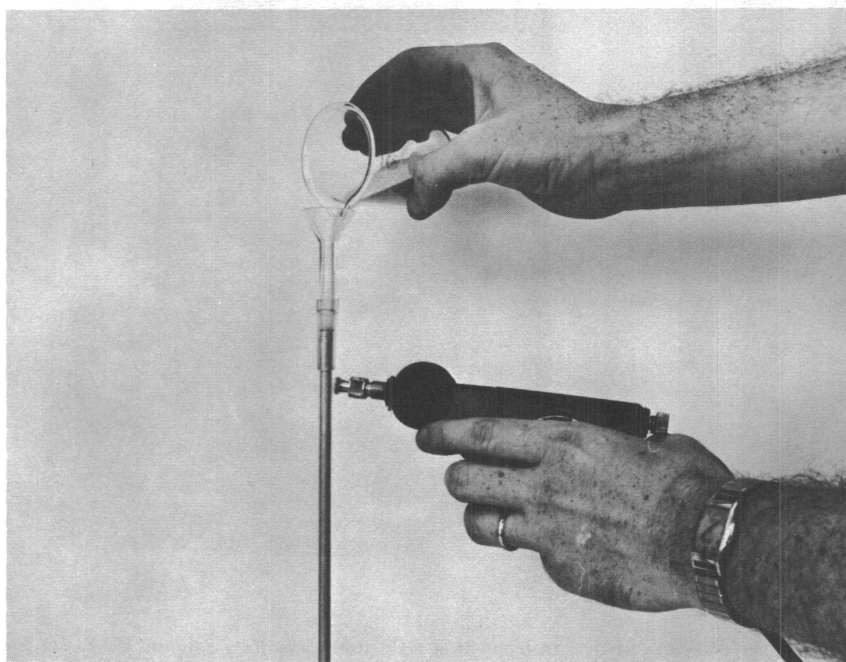
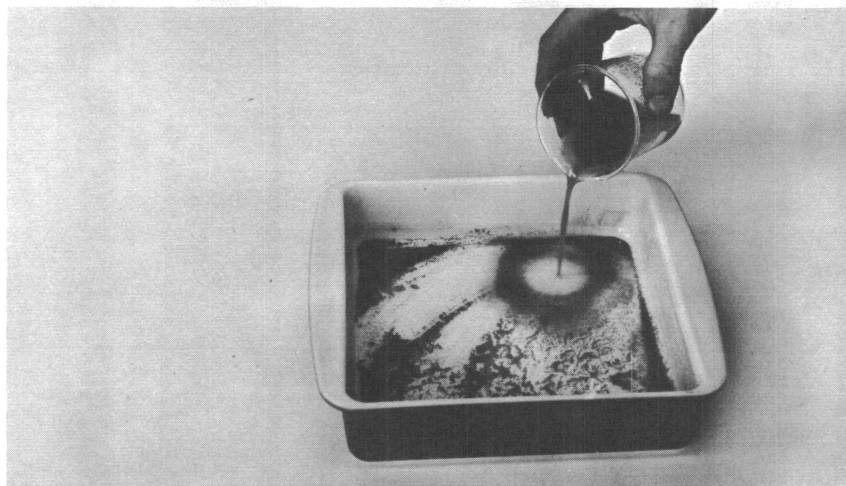
SAMPLE IS INJECTED into a gas chromatograph by means of a syringe that can deliver liquid samples as small as one hundred-thousandth of a cubic centimeter. Gas samples require a special valve arrangement to meter the sample through a chamber of known volume.



TWO TYPES OF GAS CHROMATOGRAPH differ in the design of the column where fractionation takes place. The instrument at left uses the original type of column, a quarter-inch in diameter and one to several meters long, packed with a pulverized inert substance. The column illustrated is four meters long and is folded



twice. Instrument at right uses a capillary column, 150 to 300 feet long, without packing, proposed by Marcel J. E. Golay, a consultant to the Perkin-Elmer Corporation. A nonvolatile liquid carried on the packing, or on the inside wall of the capillary tube, acts as a partitioning agent and promotes fractionation of the sample.



PREPARATION OF CHROMATOGRAPHIC COLUMN begins (top) with pulverized diatomaceous earth, to which is added a viscous partitioning liquid dissolved in a volatile carrier. The solvent is evaporated by heat while the coated powder is gently agitated (middle). The dried coated powder is packed into a tube with the aid of a vibrator (bottom).

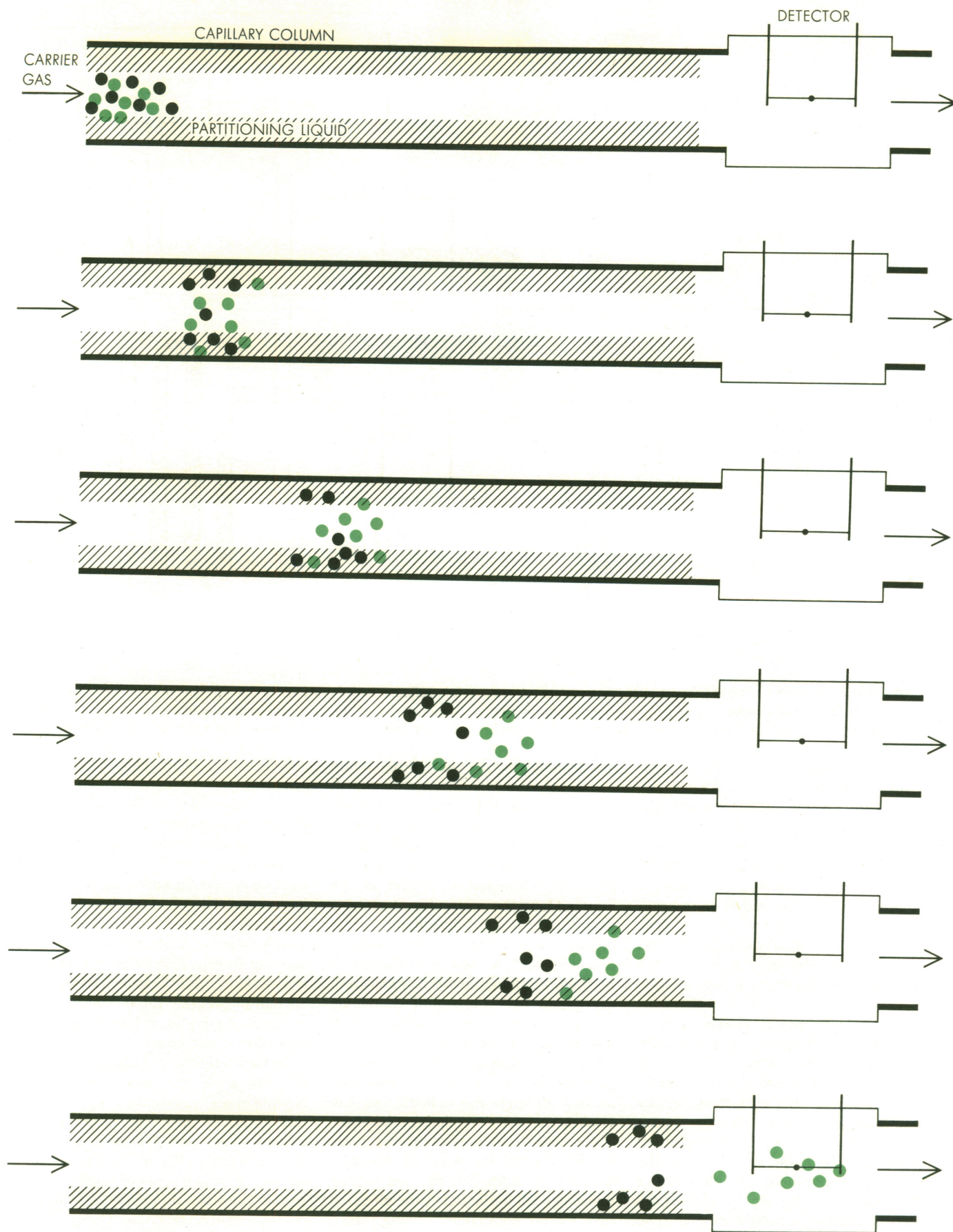
ferent substances have been successfully analyzed in one pass. Analysis time is typically a few minutes and sometimes only a few seconds. Samples usually range in size from a few hundredths to a few thousandths of a gram. Some instruments can handle samples weighing not much more than a millionth of a gram, and in such samples they can detect the presence of substances that weigh no more than a trillionth of a gram—about the weight of a single bacterium.

The fractionating column of a typical gas chromatograph consists of a copper or stainless steel tube about a quarter of an inch in diameter and from one to four meters long, though occasionally much longer tubes are used. The tube is packed with an inert material such as firebrick or diatomaceous earth that has been pulverized and coated with a non-volatile liquid called a partitioner [see illustration at left]. After the tube has been packed it is usually bent into a series of U turns or wound into a helix so that it can be fitted easily into an insulated box, the temperature of which is thermostatically controlled.

The liquid selected as the partitioner largely determines the performance of the chromatograph. The partitioner must not react with the sample being analyzed and it must not be volatilized by the stream of carrier gas that propels the sample through the column. Above all, the partitioner must show different affinities (to use an old-fashioned term) for each of the substances likely to be found in the sample mixture.

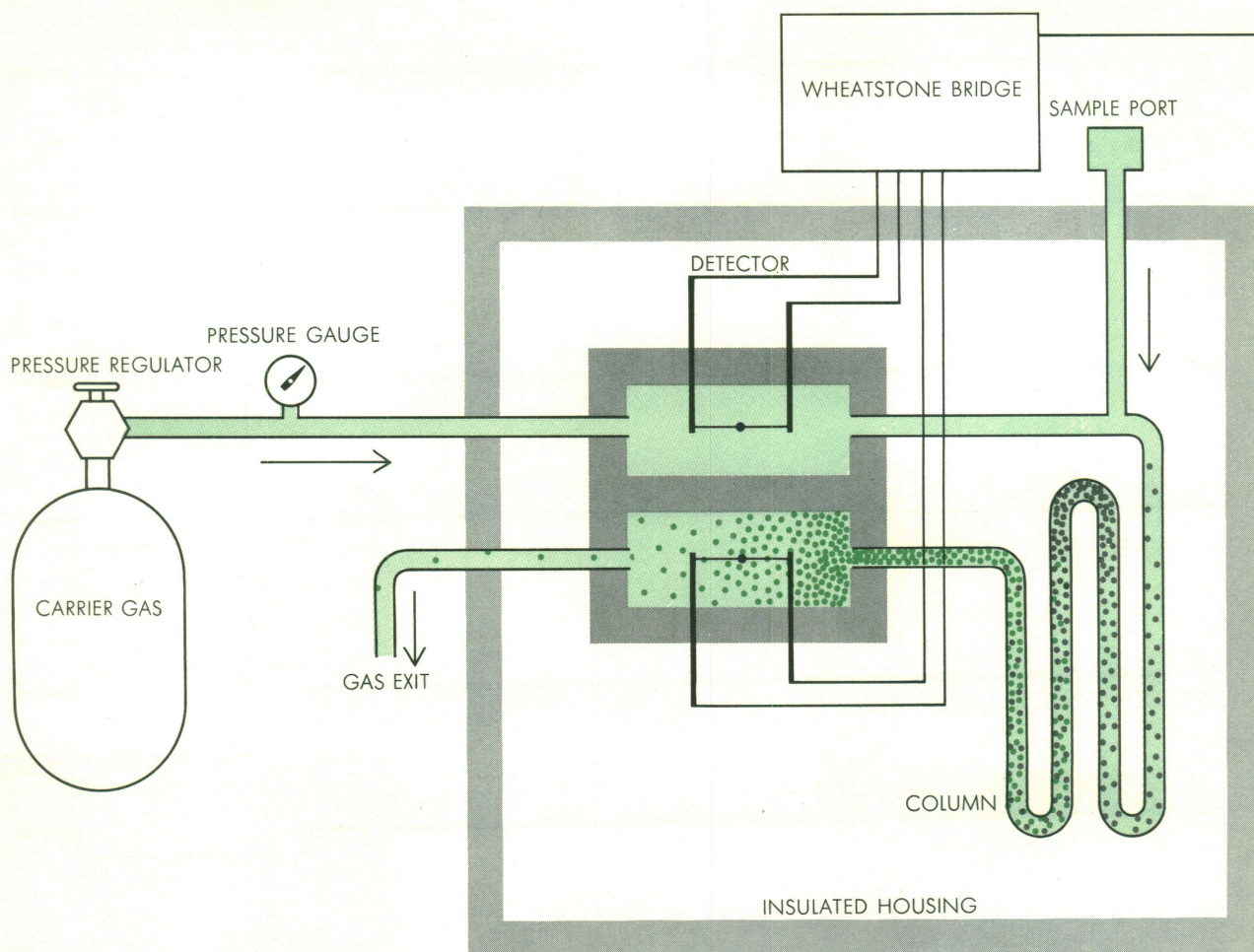
Partitioners that work well with one type of sample may be completely useless for another type. As the sample is moved through the column by the carrier gas, the partitioner must interfere in a selective fashion with the progress of each compound present, slowing up the progress of some and letting others travel through the column more swiftly. At the outlet of the column a detecting device signals the emergence of each different compound by activating a recording pen on a strip chart.

In the search for good partitioners, builders of gas chromatographs have experimented with virtually every viscous fluid, grease and low-melting-point solid in the laboratory, including such substances as silicone rubber, stopcock grease and hydrogenated shark oil. This eclecticism has sometimes had unhappy results when excellent separations were achieved with substances that could never be duplicated, even when re-



CHROMATOGRAPHIC SEPARATION takes place when a sample mixture (black and colored balls) is driven by an inert gas through a capillary tube coated with an immobile liquid called a partitioner. In the original form of gas-liquid chromatography the partitioner

was deposited on a pulverized packing. The role of the partitioner is to dissolve (and adsorb) various components of the sample in differential fashion. After fractionation the separated components pass through a detector, whose output is recorded on a chart.



SIMPLICITY OF GAS CHROMATOGRAPH is among the virtues of the instrument. The sample is swept by a carrier gas through a specially treated column in which various components of the sam-

ple migrate at different speeds. A detector measures the electrical conductivity of the gas leaving the column as well as that of the carrier gas entering the column. The difference, as determined by

ordered by the same lot or batch number. In order to deposit the viscous partitioner on the pulverized support, the partitioner is usually dissolved in a volatile solvent that can be evaporated, leaving the partitioner behind. Normally the partitioner weighs from about a quarter to a third as much as the pulverized packing in the column. The coated particles should flow freely, and there is some advantage in holding the amount of partitioner to a minimum.

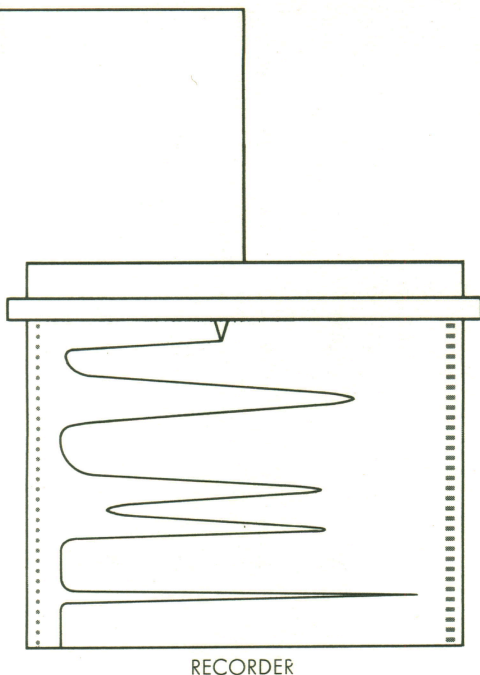
Let us now look more closely at what happens to a collection of molecules injected into the carrier gas moving through the column of a gas chromatograph. Some of the molecules rapidly dissolve in the liquid partitioner, and a dynamic equilibrium is soon established as they pass back and forth between the liquid and vapor filling the interstices of the column packing. At equilibrium the concentration of molecules of each type is a constant ratio in the two phases. Molecules of compound A, for example, may partition themselves equally between the liquid and vapor phase; molecules of compound B, on the other hand,

may be highly soluble in the liquid phase and therefore relatively few of them will be found in the vapor phase once equilibrium is reached. In this case the moving gas will tend to sweep molecules of compound A down the tube, leaving those of compound B behind in the liquid. Once the molecules of A have been carried to a region containing fresh liquid, however, some of them will redissolve until a new equilibrium is reached. By the same token, when fresh gas passes over the liquid containing molecules of B in solution, some of the B molecules will enter the gas phase in order to establish equilibrium. If we regard the sample as a plug of molecules moving through the column, we can visualize a sort of molecular leapfrog. The volatile molecules are continuously being swept to the head of the plug, where they redissolve; the less volatile molecules fall to the tail of the plug, but they too are continuously being picked up and inched forward by the gas stream pressing from behind. Eventually, if conditions are right, all the molecules of the more volatile components will be carried clear ahead of those of the less

volatile and a clean separation will be achieved.

A major problem in the early days of gas chromatography was to find a detector that would respond quickly as the separated compounds emerged in rapid succession from the end of the fractionating column. The job of the detector is not to identify the emerging compound but merely to signal when the output gas is carrying foreign molecules and when it is not. Once this is known it is easy enough to calibrate the output readings by feeding samples of known composition into the instrument.

In their original gas chromatography of fatty acids or amines (which are organic bases) James and Martin passed the output of their column through a solvent that extracted the acids or amines from the effluent gas. The collecting was done in a series of small batches so that the time of emergence from the column could be recorded. Using a color indicator, James and Martin could tell when an acid or an amine emerged from the column. They then titrated each sample to determine how much acid or base was present. The job not only was



a Wheatstone bridge, is recorded on a strip chart. The instrument can be calibrated by analyzing samples of known composition.

laborious but also it lent itself to titratable samples only.

A much simpler and more universal solution to the detection problem was finally found in the thermal-conductivity cell. The cell utilizes the fact that the electrical resistance of a heated wire varies with its temperature. If a gas of constant composition and flow rate is allowed to pass over a heated wire, the wire will be cooled a constant amount and so register a constant resistance. If a gas of different thermal conductivity appears in the stream striking the wire, the wire will change in temperature and in electrical resistance, and this change can be recorded in ink on a strip chart.

At least two other detection devices have been developed for gas chromatography. In both devices a change in gas composition is signaled by a change in the ionization—and hence the electrical conductivity—of the gas stream. In one device the gas stream is ionized (broken up into electrically charged fragments) by being passed through a hydrogen flame; in the other device the stream is ionized by bombardment with radiation from a bit of radioactive material.

To obtain sharply defined gas-chromatograph records, called fractograms, the instrument designer can vary the pressure and flow rate of the carrier gas, the operating temperature of the column, the structure and particle size of the column packing and, of course; the nature of the liquid partitioner. A considerable body of theory and empirical art has grown up around the solute-solvent interactions that underlie effective partitioning.

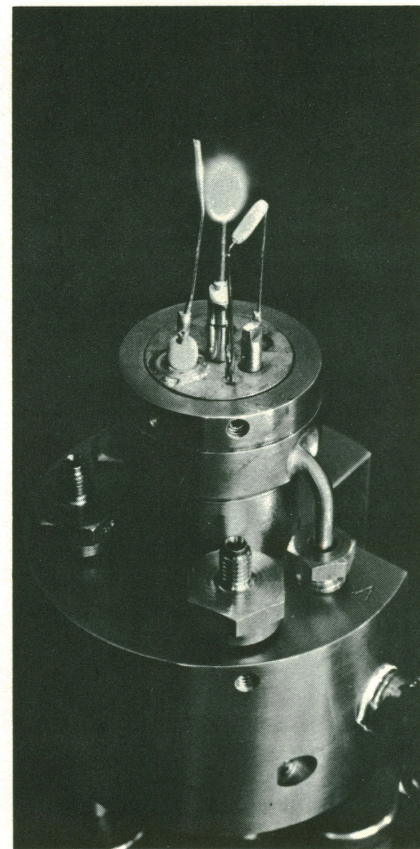
For example, if one wishes to separate a hydrocarbon and an alcohol having nearly the same boiling point (e.g., 3-methylpentane and methyl alcohol, both of which boil at about 64 degrees centigrade), it is desirable to use a partitioner that resembles the alcohol in containing hydroxyl (OH) groups in its structure. The hydrogen atom of the hydroxyl group in the alcohol will tend to form a bond with the oxygen atom of the hydroxyl group in the partitioner. The hydrocarbon will not form such a bond and will therefore move through the column faster. A relatively nonvolatile liquid containing hydroxyl groups that can be used for this separation is polyethylene glycol.

Offhand one might think that a partitioner, to be effective, should show a differential solvent action on each component in a sample mixture. One might then conclude that the solutes would be retained by a partitioner in order of their solubility. In practice, however, this is not always true. The reason is that solubility as conventionally measured with solvents in bulk—say in a laboratory beaker—is quite different from the solubility shown when a solvent is thinly distributed over an enormous surface area, as it is in a chromatographic column. In the latter case a new factor appears: the effect of surface adsorption. A solute is said to be adsorbed if its concentration in the immediate region of the surface exceeds that in the bulk liquid. Adsorption can arise either at the interface between the solid support and the partitioning liquid, or at the interface between liquid and gas, or it can occur in both regions. Sometimes adsorption enhances the desired separation; at other times it interferes with it. For example, when alcohols are chromatographed on a hydrocarbon partitioner, they tend to displace the hydrocarbon and fasten themselves to the support.

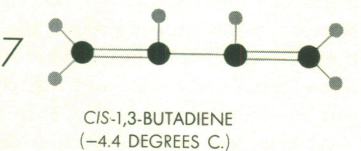
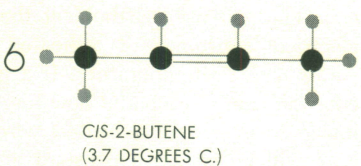
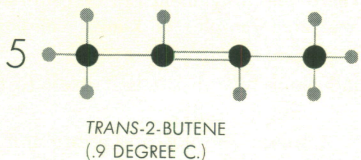
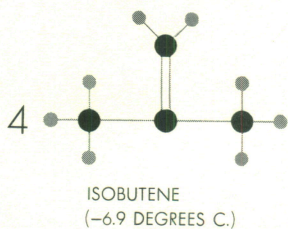
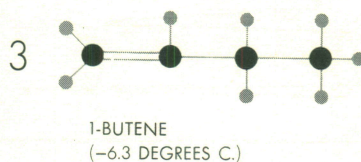
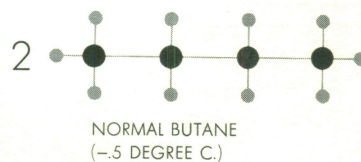
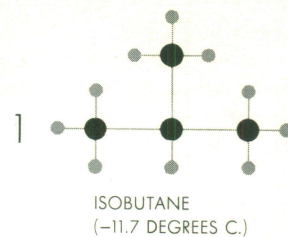
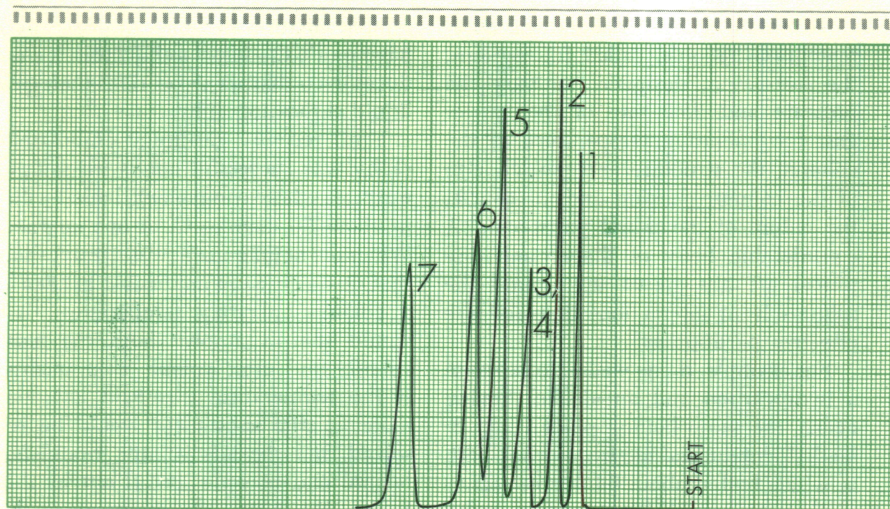
Considerable work has been done on gas chromatographs that achieve separations strictly by differential adsorption on the solid packing, without help from a liquid partitioner. This is called gas-solid chromatography, and for certain

sample mixtures in which the molecules have much the same architecture it produces even sharper separations than gas-liquid chromatography.

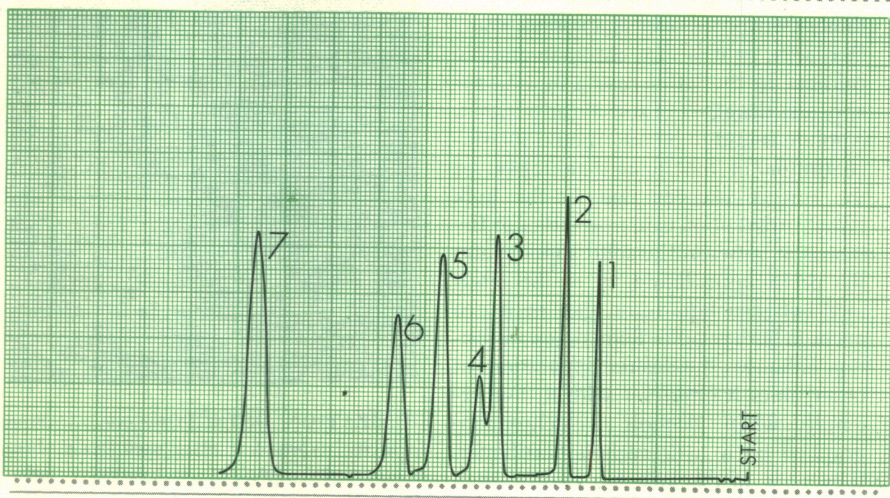
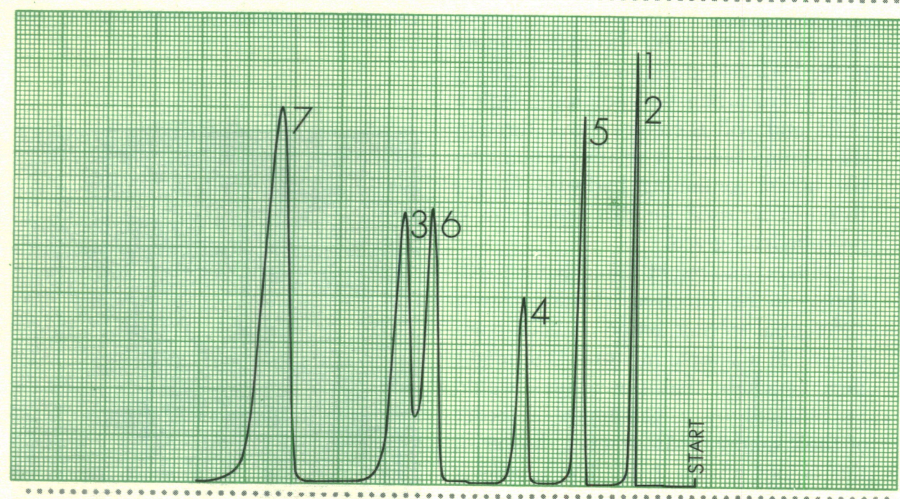
Until recently gas-liquid chromatography was successful only with sample mixtures whose components boiled within about 50 degrees C. of one another. When the boiling range was greater than that, it was usually impossible to choose a column operating temperature that would sharply resolve both the least volatile and most volatile components. If the temperature was held low, the more volatile substances would be resolved, but the less volatile would lag behind and become spread out by diffusion. By raising the temperature the resolution of the less volatile could be sharpened, but the more volatile would then rush through the column and emerge in a poorly resolved bunch. The



IONIZATION DETECTOR, one of three principal types of detector used in gas chromatography, employs a hydrogen flame to break up chemical compounds into electrically charged fragments (ions). By measuring the electrical conductivity of the ionized gas at the column exit the detector signals the passage of various fractions of the sample. In this photograph the cover of the detector has been removed and the normally colorless flame has been made visible.



● CARBON ● HYDROGEN



SEPARATION OF FOUR-CARBON HYDROCARBONS, which boil within a narrow range, is a familiar problem in the oil and synthetic rubber industries. The molecular structure and boiling points of the principal four-carbon hydrocarbons appear at right. The fractograms (left) show how these compounds are fraction-

ated by different partitioning liquids: dimethylsulfolane (top), silver nitrate in diethylene glycol (middle) and hexanedione (bottom). The upper two fractograms were made on columns four meters long operated at 25 degrees centigrade. Bottom fractogram was made on a column two meters long operated at zero degrees.

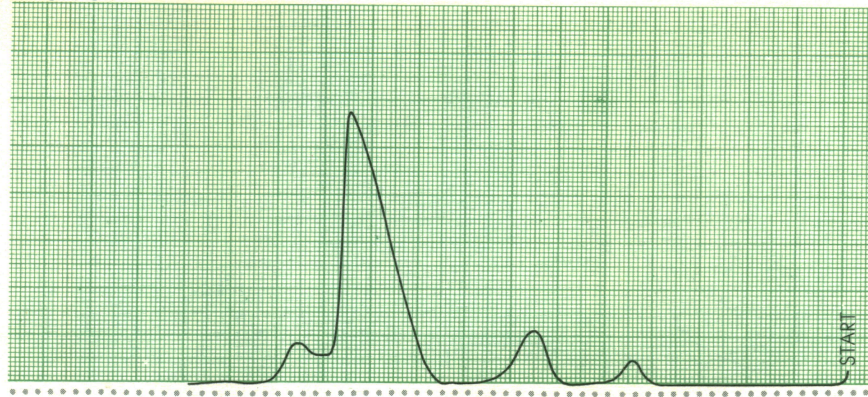
answer to this problem has been found in "temperature programing," which simply means starting the separation at a low temperature and raising it in regular steps until the job is done. By this procedure one can fractionate mixtures whose components have a boiling range as broad as 200 degrees C.

Actually it is not necessary for the components of a sample to be anywhere near their boiling points for gas chromatography to work. At the University of Arizona we have analyzed samples of volatile inorganic halides (for example, niobium chloride) at temperatures near their melting point, or in the vicinity of 250 degrees C. Other investigators have operated machines at 500 degrees C. that will chromatograph compounds boiling as high as 625 degrees C.

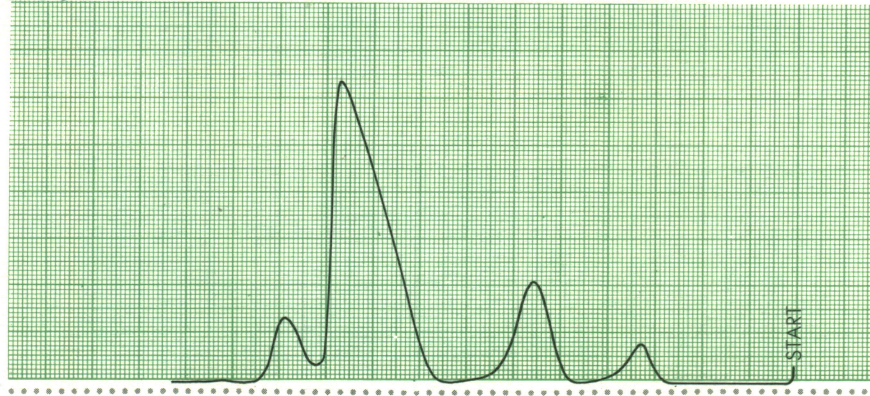
An important advance in the design of gas chromatographs was made about 1956 by Marcel J. E. Golay, a consultant to the Perkin-Elmer Corporation. Golay, a student of information theory, conceived the improvement after making a theoretical study of the migration of a solute through a packed column. In the fashion characteristic of theorists he sought a simplified model to substitute for the actual highly complex situation prevailing in a tube packed with porous particles of random shapes and sizes. He selected for his model a bundle of capillary tubes, equal in diameter to the granule size of the packing, which were evenly coated with the partitioning medium. Upon analyzing his calculations Golay concluded that a single, very long coated capillary tube would achieve separations equivalent, if not superior, to those produced by packed columns and do so in a much shorter time and under less severe conditions of temperature and driving pressure. Golay met his skeptics by preparing some of the first columns himself. They amply fulfilled his predictions.

Commercial columns of his design usually employ a capillary tube having an inside diameter of .01 inch. The tubes, ranging from 150 to 1,000 feet in length, are coiled into a compact helix [see illustration at right on page 3]. If these fine capillary columns are not to be flooded, the sample size must be extremely small, usually about five-millionths of a cubic centimeter in liquid volume, or about five-thousandths of a cubic centimeter after vaporization. To obtain such minute volumes a sample some 20 times larger is vaporized and shot through a stream splitter that ad-

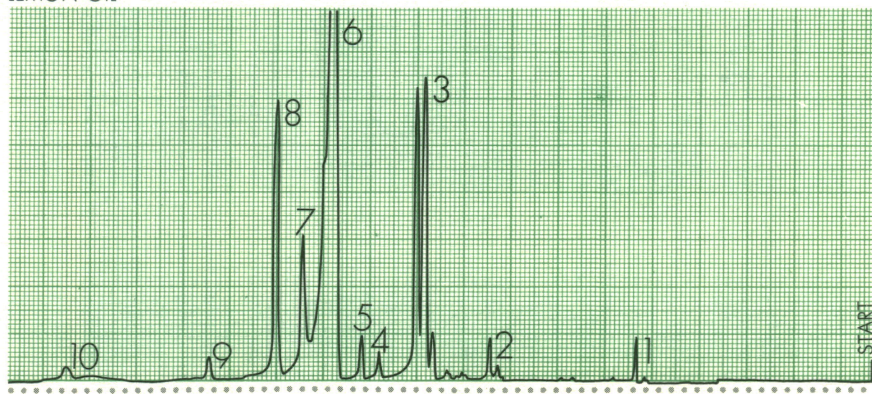
LEMON OIL



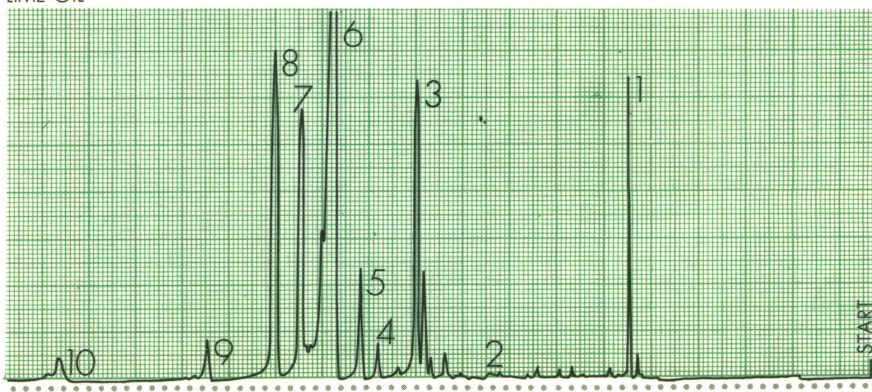
LIME OIL



LEMON OIL

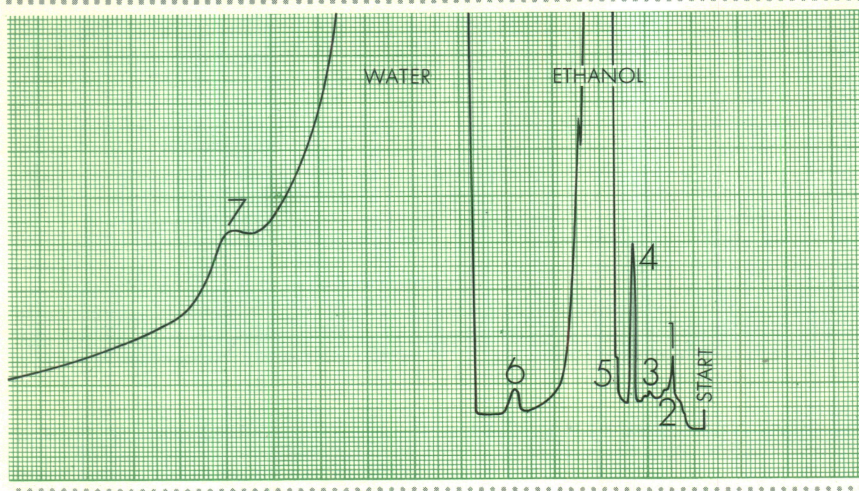


LIME OIL



PERFORMANCE OF OLD AND NEW INSTRUMENTS is demonstrated by fractograms of two similar essential oils: the terpene fractions of lemon oil and lime oil. When analyzed on a standard two-meter packed column, the two oils produced the upper pair of fractograms. When analyzed on a 175-foot capillary (Golay) column, they produced the detailed lower pair. The peaks labeled "6" are made by limonene ($C_{10}H_{16}$), which has a lemon-like odor.

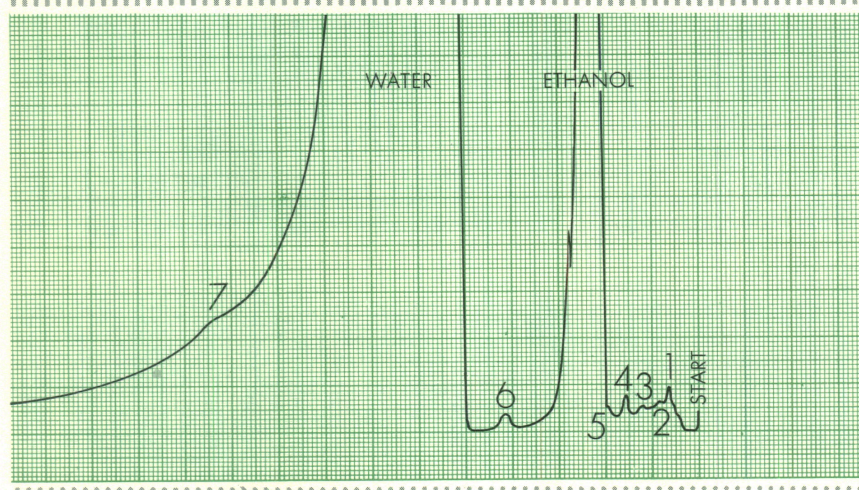
BOURBON



- 1 ACETALDEHYDE
- 2 FORMALDEHYDE
- 3 ETHYL FORMATE
- 4 ETHYL ACETATE
- 5 METHANOL
- 6 PROPANOL
- 7 ISOAMYL ALCOHOL

mits about 5 per cent to the column and discards the remainder. Ordinarily a few minutes to half an hour are required for such samples to migrate through the column. As an extreme example of what can be achieved with a capillary column and a highly sensitive detector, it has been possible to record 14 peaks—each representing a separate compound in a sample of closely related hydrocarbons—in less than two seconds.

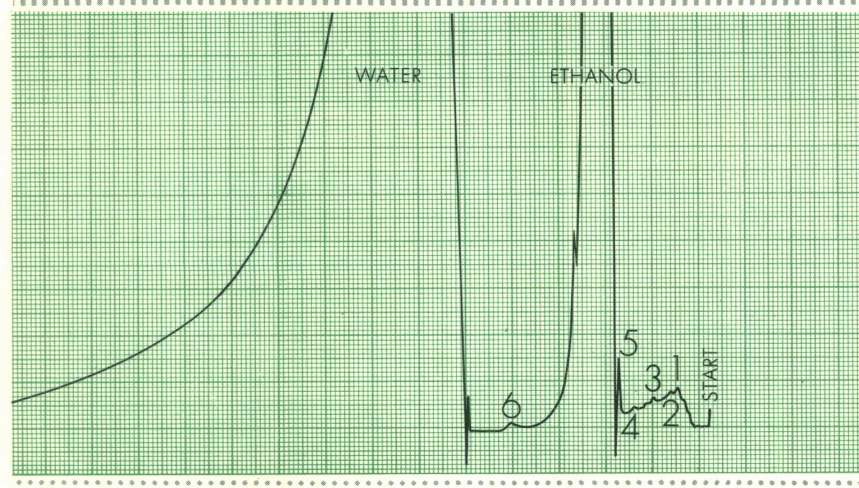
SCOTCH



The areas under different peaks in a fractogram are roughly proportional to the fractional amounts of each substance in the original sample. With care the method is accurate to about 2 per cent. In general the investigator has two methods for discovering exactly what substance is represented by a particular peak. The commonest method is to use samples of known composition to calibrate the machine. Alternatively he may isolate the column effluent that produced a given peak and characterize it by some suitable analytical technique, for example by using an infrared spectrophotometer or a mass spectrometer. The gas chromatograph will separate not only compounds with closely similar properties but also various forms of individual compounds. In organic chemistry almost all but the simplest compounds can exist in two or more forms known as isomers. These are molecules containing the same number and kind of atoms fitted together in different geometrical arrangements [see illustration on page 8].

In some cases gas chromatography provides direct clues to compound identification. The volume of gas, called the retention volume, that precedes a particular solute through the column depends on the nature of the solute, the choice of partitioner and the temperature. Within limits, retention volume is not overly sensitive to length of column, driving pressure, flow rate or the amount of partitioner employed. As a result one can determine retention volumes for various compounds of interest and use these volumes for identifying unknown samples [see illustration on opposite page]. A considerable effort is now being made to utilize gas chromatography for determining the structure of molecules by

GIN



FRACTOGRAMS OF POTABLE SPIRITS provide a sensitive measure of volatile components that influence taste and aroma. These analyses confirm that Scotch and gin contain fewer such components than bourbon. (Isoamyl alcohol is fusel oil.) Samples were run on a two-meter packed column. Peaks were identified by adding various known compounds to a whiskey that had previously been analyzed. Analyses were made by Robert B. Carroll, a chemical consultant, and Lawrence C. O'Brien, then at the Perkin-Elmer Corporation.

relating retention volume to particular molecular configurations.

Much of the explosive growth of gas chromatography can be attributed to the petroleum industry, which has to deal with materials of extraordinary complexity. Crude oils commonly contain more than 150 different hydrocarbons, many of them isomers of each other. For separating them gas chromatography has proved a powerful tool. Oil firms have now begun to use gas chromatographs for continuous monitoring of process streams in the refinery. Chromatographic analyses will be transmitted to a computer, which will automatically calculate the optimum operating conditions for catalytic cracking towers. Gas chromatography is almost the only method available for detecting certain catalytic poisons that impair the polymerization process when present in concentrations of only 50 parts per million.

Automotive engineers use gas chromatography for analyzing the exhaust of engines under a variety of operating conditions with various fuels. The results are used to improve both engines and fuels and also contribute to a reduction in the air pollution created by engine exhausts.

Elsewhere, fractograms are rapidly replacing the opinions of "sniff and taste" panels as a method of assaying the uniformity of instant coffees, blends of whiskey and many other products whose commercial acceptance depends on subtle flavors and aromas. In such products gas chromatography can often detect trace components present in only one part per billion. It is, in fact, the first analytical instrument to rival the human nose in sensitivity.

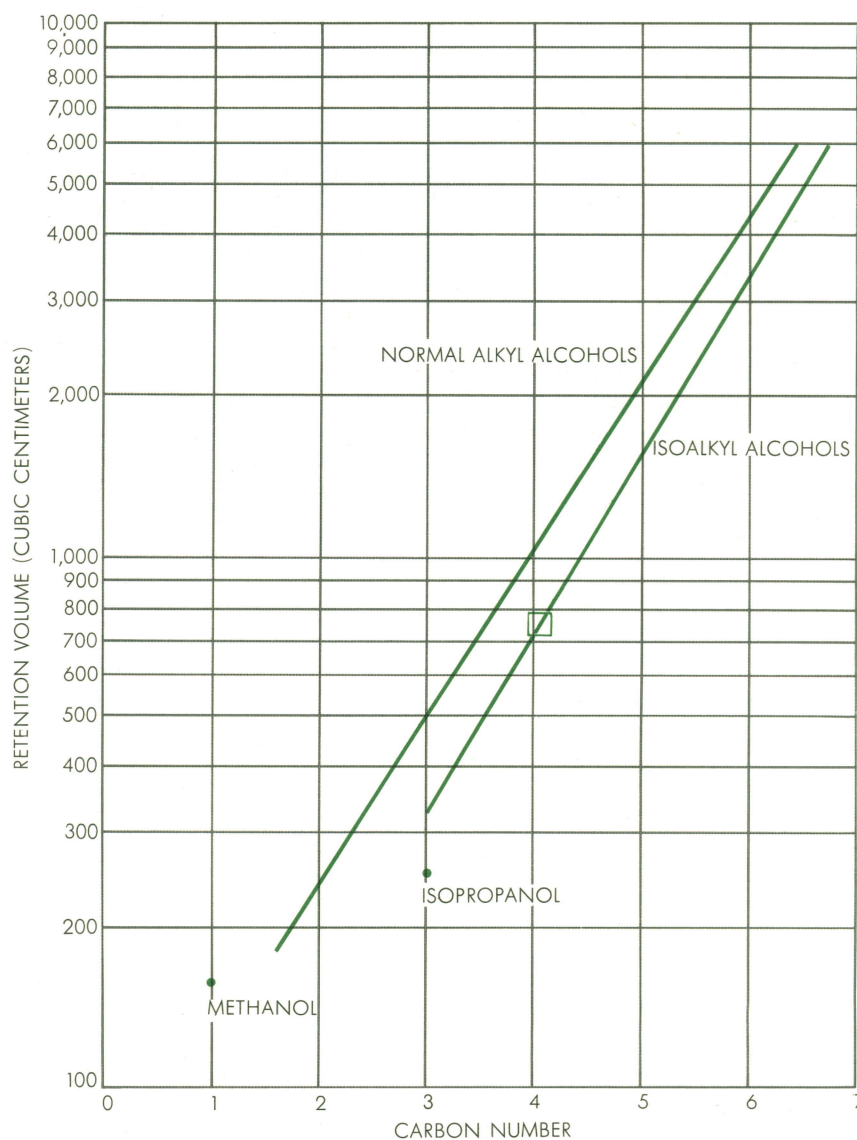
Before long one can reasonably expect that gas chromatography will be used not only as an analytical tool but also as a method of preparing ultrapure materials. Columns are now operating that can refine and separate the components in batches about one ounce in size. If the process can be made continuous, it should be able to provide laboratory chemicals and even commercial compounds having a wholly new order of purity.

Recently Donald E. Johnson, Sara Jo Scott and Alton Meister of Tufts University announced success in a long-sought goal: separation by gas chromatography of derivatives of the amino acids. The method may prove superior to the liquid-solid chromatographic method, employing a column packed with ion-exchange resins, that has been widely used for the analysis of the amino acids in proteins [see "The Chemical

Structure of Proteins," by William H. Stein and Stanford Moore; *SCIENTIFIC AMERICAN*, February, 1961].

A gas chromatograph of remarkable capabilities is now being developed by the Aerojet-General Corporation and the Jet Propulsion Laboratory of the California Institute of Technology; it will be placed aboard the *Surveyor* moon probe scheduled for launching in 1963. Upon reaching the moon the probe will pick up samples of lunar crust, grind them and feed them into a heater, where they will be pyrolyzed. The gas chromatograph will include two types of detector to analyze samples pyrolyzed at 150, 325, 500 and 1,000 degrees C. The heat-

er and chromatograph must consume no more than 10 watts of current. The whole apparatus must weigh no more than 11.5 pounds and must fit inside a box measuring 8 by 8 by 10 inches. Finally, to withstand the shock of landing on the moon, it must tolerate a deceleration force equivalent to 100 times the force of gravity on the earth. An important objective of the lunar chromatograph is to learn whether or not the moon's crust contains complex organic compounds of the type associated with living matter. This is an impressive assignment for an analytical tool that has come into general use only within the past five or six years.



COMPOUND IDENTIFICATION is often possible with gas chromatography when the sample is known to contain one of a limited number of compounds. For example, normal alkyl alcohols, whose carbon atoms form a straight chain, require a greater volume of carrier gas to drive them through a given column than isoalkyl alcohols having the same number of carbon atoms in a branched chain. Thus if an unknown four-carbon alcohol has a retention volume of 750 cubic centimeters of carrier gas (*square*), one can be sure it is isobutanol and not normal butanol, which would have a retention volume of about 1,000 c.c.

The Author

ROY A. KELLER is assistant professor of chemistry at the University of Arizona. Born in Davenport, Iowa, in 1928, he received a B.Sc. from Arizona in 1950 and an M.S. in chemistry in 1952. Keller then went to the University of Utah, where he studied under the direction of Henry Eyring. It was Eyring who first suggested that Keller do research in chromatography. Keller obtained his Ph.D. from Utah in 1956 and joined the faculty of Arizona later that year.

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